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The assembly and evaluation of antisense oligonucleotides applied in exon skipping for titin-based mutations in dilated cardiomyopathy.

Hahn JK¹, Neupane B², Pradhan K², Zhou Q¹, Testa L³, Pelzl L⁴, Maleck C¹, Gawaz M¹, Gramlich M⁵.

Author information

Abstract

The leading cause of genetic dilated cardiomyopathy (DCM) is due to mutations in the TTN gene, impacting approximately 15-20% of familial and 18% of sporadic DCM cases. Currently, there is potential for a personalized RNA-based therapeutic approach in titin-based DCM, utilizing antisense oligonucleotide (AON) mediated exon-skipping, which attempts to reframe mutated titin transcripts, resulting in shortened, functional protein. However, the TTN gene is massive with 363 exons; each newly identified TTN exon mutation provides a challenge to address when considering the potential application of AON mediated exon skipping. In the initial phase of this strategy, the mutated TTN exon requires specific AON design and evaluation to assess the exon skipping effectiveness for subsequent experiments. Here, we present a detailed protocol to effectively assemble and evaluate AONs for efficient exon-skipping in targeted TTN exons. We chose a previously identified TTN 1-bp deletion mutation in exon 335 as an exemplary target exon, which causes a frameshift mutation leading to truncated A-band titin in DCM. We designed two specific AONs to mask the Ttn exon 335 and confirmed successfully mediated exon skipping without disrupting the Ttn reading frame. In addition, we evaluated and confirmed AON-treated HL-1 cells show maintained store-operated calcium entry, fractional shortening as well as preserved sarcomeric formation in comparison to control samples, indicating the treated cardiomyocytes retain adequate, essential cell function and structure, proving the treated cells can compensate for the loss of exon 335. These results indicate our method offers the first systematic protocol in designing and evaluating AONs specifically for mutated TTN target exons, expanding the framework of future advancements in the therapeutic potential of antisense-mediated exon skipping in titin-based DCM.

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KEYWORDS: Dilated cardiomyopathy; Exon skipping; Titin

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